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Filed : May 3, 2002

REMARKS

Applicants thank the Examiner for the review of the instant application. Claims 6-17 remain pending and are presented for further examination. For the reasons stated below, Applicants respectfully traverse the rejection of the pending claims.

Rejection Under 35 U.S.C. §101

The PTO maintains its rejection of Claims 6-17 under 35 U.S.C. § 101 as lacking a specific and substantial asserted utility or a well established utility for the reasons set forth in the previous Office Actions. The PTO asserts that one skilled in that art would not assume that an increase in mRNA expression would correlate with significantly increased polypeptide levels, and that “the specification fails to precisely disclose any correlation between the reported overexpression of PRO1268 mRNA and PRO1268 protein expression, and more importantly, to what extent PRO1268 mRNA is reliably overexpressed in a particular tumor sample, such as kidney, such that the PRO1268 polypeptide encoded thereby could be used as a diagnostic marker for kidney tumors. There is no evidence regarding whether or not PRO1268 polypeptide levels are overexpressed in kidney tumors.” *Final Office Action* at 10.

For the reasons set forth below, Applicants respectfully disagree.

Applicants incorporate by reference their previously submitted arguments, and for the reasons of record assert that the specification contains a disclosure of utility and therefore must be taken as sufficient to satisfy the utility requirement of 35 U.S.C. § 101. Applicants also submit that for reasons of record, the Examiner has not met the PTO’s burden of providing evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility. However, even if the Examiner has met the PTO’s initial burden, Applicants’ rebuttal evidence previously submitted and additional evidence submitted herewith is sufficient to prove that it is **more likely than not** that a person of skill in the art would be convinced, **to a reasonable probability**, that the asserted utility is true. As stated previously, Applicants’ evidence need not be direct evidence, so long as there is a reasonable correlation between the evidence and the asserted utility. **The standard is not absolute certainty.**

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Substantial Utility

Summary of Applicants' Arguments and the PTO's Response

Applicants remind the PTO that the asserted utility rests on the following argument:

1. Applicants have provided reliable evidence that mRNA for the PRO1268 polypeptide is expressed at least two-fold higher in kidney tumor tissue compared to normal kidney tissue;
2. Applicants assert that it is well-established in the art that a change in the level of mRNA for a particular protein, e.g. an increase, generally leads to a corresponding change in the level of the encoded protein, e.g. an increase;
3. Given Applicants' evidence that the mRNA for the PRO1268 polypeptide is differentially expressed in kidney tumor tissue compared to normal kidney tissue, it is more likely than not that the PRO1268 polypeptide is likewise differentially expressed in these tumors; the PRO1268 polypeptide is therefore useful as diagnostic tools to distinguish kidney tumor tissue from normal kidney tissue.

Applicants understand the PTO to be making the following arguments in response to Applicants' asserted utility:

The PTO continues to rely on several references of record, as well as newly produced references by Nagaraja *et al.* (Oncogene, (2006) 25:2328-38), Waghray *et al.* (Proteomics, (2001) 1:1327-38) and Sagynaliev *et al.* (Proteomics, (2005) 5:3066-78) to support its position that changes in the level of mRNA do not necessarily reflect changes in protein expression levels. The PTO argues that the Nagaraja *et al.*, Waghray *et al.* and Sagynaliev *et al.* references support the idea that "increases in mRNA and protein samples are not correlated." *Final Office Action* at 6.

The PTO also states that Applicants previously submitted references are not persuasive with regard to the instant application and the question of whether changes in mRNA levels correlate with changes in protein levels. According to the PTO, Applicants' exhibits, with the exception of Orntoft *et al.* (Mol. Cell. Proteomics, (2002) 1:37-45), "measure mRNA, while the assay utilized in Example 18 of the instant specification is PCR." *Final Office Action* at 4. In addition, the PTO states that with the exception of Fletcher *et al.*, all of the newly cited references are only directed towards a single gene or a small number of genes. Thus, in regard to the correlation between changes in mRNA levels and changes in protein levels, these exhibits "are

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not found persuasive in view of comprehensive studies where significantly larger numbers of transcripts and proteins were examined and which accurately describe general trends, specifically: Haynes (80 proteins examined), and Chen (165 proteins examined)...and Nagaraja et al. (2006), Waghray et al.(2001) and Sagynaliev et al. (2006)....” *Final Office Action* at 5.

Applicant’s maintain that in light of all of the evidence, the PTO’s arguments are not adequate to support the utility rejection of the claimed invention under 35 U.S.C. § 101.

The PTO has Concluded that the data in Example 18 are Sufficient to Establish the Utility of the Claimed Invention

As an initial matter, Applicants point out that in other applications filed by Applicants that rely on data from the exact same disclosure, *Example 18*, and in which Applicants have submitted substantially the same references in support of their asserted utility, the PTO has concluded that: “[b]ased on the totality of evidence of record, **one of skill in the art would find it more likely than not that an increase in message as measured by RTPCR would be predictive of an increase in protein expression levels**, absent evidence to the contrary. Therefore, the data presented in *Example 18*, which demonstrates differential expression of nucleic acids encoding PRO1180, also supports a conclusion of differential expression of PRO1180 polypeptide. Therefore, one of ordinary skill in the art would be able to use the PRO1180 polypeptide diagnostically for distinguishing normal kidney and rectal tumor tissues compared to kidney tumor and normal rectal tissue, as asserted by Applicant.” See *Examiners Reasons for Allowance* in pending Application No. 10/063,529. See also *Examiners Reasons for Allowance* in Application No. 10/063,530, No. 10/063,524, No. 10/063,582; and No. 10/063,583, all of which conclude that the data presented in *Example 18*, which demonstrate differential expression of the nucleic acids encoding certain PRO polypeptides, also support a conclusion of differential expression of the PRO polypeptides, making the claimed PRO polypeptides and antibodies that bind the PRO polypeptides useful for diagnostic purposes.

Applicants therefore request that the Examiner recognize the utility of the claimed invention, supported by the data presented in *Example 18* and Applicants numerous cited references, as was done in the other applications referenced above.

The Previously Cited References Provide Evidence that Changes in mRNA Levels are Correlated with Changes in Protein Levels

Applicants turn to the PTO's argument that the evidence of differential expression of the gene encoding the PRO1268 polypeptide in kidney tumor tissue compared to normal kidney tissue is insufficient in view of the teachings of Hu *et al.*

Applicants incorporate by reference their previously submitted arguments in regard to Hu *et al.* and will not reiterate those arguments here. However, Applicants will once again explain why the PTO's reliance on Hu is misplaced. Hu bases his conclusions on data generated from high throughput microarrays:

In any microarray experiment, thousands of genes may demonstrate statistically significant expression changes, but only a fraction of these may be relevant to the study. Hu at 405, left column, first paragraph (emphasis added).

As Applicants previously pointed out, Applicants are relying on a more accurate and reliable method of assessing changes in mRNA level, namely quantitative PCR analysis. Applicants submitted a reference by Kuo *et al.*, (Proteomics 5(4):894-906 (2005)), in which the authors state that PCR is a "more reliable and sensitive" than microarray technology. Kuo *et al.* at Abstract (emphasis added). Thus, even if accurate, Hu's statements regarding microarray studies are not relevant to the instant application which does not rely on microarray data.

Applicants maintain that Kuo supports their assertion that Applicants' PCR data are more accurate and reliable than the microarray data relied on by Hu. Because PCR is more accurate and reliable than microarrays, conclusions regarding the relevance of mRNA transcript changes based on microarray data, such as those set forth in Hu, are not applicable to data generated using the more reliable method. Kuo supports this assertion because it is evidence that one of skill in the art would regard PCR as a more accurate and reliable method of assessing changes in mRNA.

The PTO's Newly Cited References (Nagaraja et al., Waghray et al. and Sagynaliev et al.) Do Not Support the Conclusion that Changes in mRNA Expression Do Not Result in Changes in Protein Expression

Applicants continue to assert that it is well-established in the art that a change in the level of mRNA encoding a particular protein generally leads to a corresponding change in the level of

the encoded protein; given Applicants' evidence of differential expression of the mRNA for the PRO1268 polypeptide in kidney tumors, it is more likely than not that the PRO1268 polypeptide is also differentially expressed; and proteins differentially expressed in certain tumors, and antibodies that bind such proteins, have utility as diagnostic tools.

In response to Applicants' assertion, the PTO cites new references by Nagaraja *et al.* (Oncogene, (2006) 25:2328-38), Waghray *et al.* (Proteomics, (2001) 1:1327-38) and Sagynaliev *et al.* (Proteomics, (2005) 5:3066-78) as support for the argument that "increases in mRNA and protein samples are not correlated." *Final Office Action* at 6.

The PTO argues that in Nagaraja *et al.*, researchers observed that there were fewer changes observed in protein abundance as compared to transcript abundance between various malignant and normal breast cell lines and that "[t]he comparison of transcript profiles with proteomic profiles demonstrated that altered proteins were not always represented in the microarray designated profiles and *vice versa*." *Id.* at 6. The PTO sees these observations as support for its contention that mRNA levels are not predictive of protein levels, even when considering the effect of changes in mRNA levels on protein levels. However, a careful examination of Nagaraja *et al.* shows that the reference does not contain evidence that supports the PTO's position.

Nagajara and colleagues analyzed the transcriptomes and proteomes of normal and malignant breast cell lines. In the studies of the transcriptomes of these cell lines, the gene chips used in the microarray analysis could detect 18,400 gene or gene variants. Nagajara *et al.* reported over 1000 genes that had a two-fold or greater differential level of expression between the various cell lines studied. The researchers distinguished between differentially expressed transcripts that were upregulated and those that were downregulated, comparing the normal cell line to the malignant ones and the malignant ones to each other (Figure 5, pg. 2332).

However, in their proteome studies, the researchers used techniques that were far less sensitive and only able to detect a small number of proteins: "Typically, > 300 protein spots could be visualized in silver-stained gels, and there were far fewer protein spots in gels that were stained with Coomassie blue" (pg. 2332). While the gene chips used by the researchers in their transcriptome work could detect 18,400 gene and gene variant transcripts, the proteome analysis techniques used by the researchers could only detect a much smaller number of proteins.

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Evidently, the protein analysis techniques used were not sensitive enough to detect any but the most abundant proteins. As a result, the total number of proteins detected in the most sensitive protein gel used (" > 300 ") was only 1/3 of the total number of transcripts found to be differentially expressed and only about 17% of the total number of transcripts that could be identified by microarray analysis. The proteins detected do not represent a random, representative sample of proteins from the cells; instead, they actually represent a sample of only the most highly expressed and abundant proteins. Additionally, the proteins selected for identification from the gels were only those proteins that were either *upregulated* or solely detected in the malignant cell lines, as compared to the normal cell line. Proteins that were downregulated in the malignant cell lines, only expressed in the normal cell line or differentially expressed between the malignant cell lines were not studied in the proteosome analysis.

Due to the difference between the techniques used and the strategies employed by the researchers in this study, the data from the transcriptosome and proteosome studies cannot be reliably compared to one another. The transcriptosome studies examined 18,400 transcripts and variants and uncovered thousands of differentially expressed transcripts, both upregulated and downregulated. The proteosome studies only detected around 300 of the most abundant proteins in the cell lines. The researchers only selected proteins that were upregulated or solely expressed in malignant cells for study. Thus, genes expressed at a relatively low rate or gene products that are relatively less abundant were included in the transcriptosome study but excluded from the proteosome study. Additionally, different criteria were used for defining altered expression of transcripts than were used to select proteins for identification. The criteria for picking a protein for study (only those upregulated by two-fold or greater, or solely expressed, in malignant cells) was narrower than the criteria for examining differentially expressed transcripts (any transcript with a two-fold or greater upregulation or downregulation between any combination of two of the three cell lines examined). Thus, the population of genes examined in the proteosome experiments represents a small, non-random subset of the population examined in the transcriptosome experiment, both in terms of the total population of transcripts or gene products uncovered by the experiments and in terms of those particular transcripts or gene products that were identified as altered in expression. Because the genes analyzed in the proteosome experiments represent neither a similar set nor a representative, randomly selected subset of the

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genes analyzed in the transcriptosome experiments, no valid conclusions can be drawn by comparing the results from the two types of experiments to one another.

The PTO cites several sentences from Nagaraja *et al.* in support of its argument that “mRNA levels are not necessarily predictive of protein levels ... even when there is a change in the mRNA level.” *Office Action* at 6. Specifically, the PTO cites:

“...the proteomic profiles indicated altered abundance of few proteins as compared to transcript profiles...”;

“the comparison of transcript profiles with proteomic profiles demonstrated that altered proteins were not always represented in the microarray designated profiles and *vice versa*”; and

“as dictated by post-transcriptional regulation, protein profiles showed far fewer changes as compared to transcript profiles.”

However, as the above analysis of experimental techniques and design reveals, transcriptosome and proteosome study data cannot be compared to each other to draw conclusions about the relationship between mRNA levels and protein levels. This is due to a wide difference in technique sensitivity, which lead to the examination of drastically different portions of the total gene transcript or product populations, and an incompatible difference in the definition of altered expression for transcripts and proteins. Nagaraja *et al.* found that the proteomic profiles had fewer proteins with an altered abundance as compared to the transcriptosome profiles and that the same genes with altered expression patterns were not always found in both proteomic and transcriptosomic profiles. However, from the observations made during analysis of the results of transcriptosome and proteosome research, Nagaraja *et al.* drew no conclusions as to the relationship between mRNA levels and protein levels. All comments made by the authors are entirely consistent with conclusions of Sagynaliev, discussed *infra*: that there is a significant need to standardize the scientific methods of collecting, storing, retrieving and analyzing samples, as well as the querying of genetic expression data obtained through a variety of techniques. By pointing out the differences between the proteosome and transcriptosome studies, the authors were not implying that there was doubt about the relationship between mRNA levels and protein levels. Rather, they were pointing out the unreliability of any conclusions that could be drawn from comparisons between studies of differential transcriptosomes and studies of differential proteosomes.

Regarding the third statement relied upon by the PTO, the conclusions of Nagaraja *et al.* about *post-transcriptional* regulation are based on studies of a cell line that was genetically engineered in the laboratory to eliminate particular transcripts through the use of anti-sense sequence technology. In these experiments, the authors deliberately reduced certain particular transcripts and then looked at the effects on cell cultures and proteosomes at one time point (when cultures were 70-80% confluent). As explained in greater detail under Waghray *et al. infra*, sudden changes and manipulations of transcript profiles can lead to wildly fluctuating levels of gene product within cells. Additionally, the amount of time that is needed to see the difference in gene product levels caused by changes and manipulations of transcript levels can vary widely from gene to gene, from hours to multiple days. Thus, examination of only one timepoint is insufficient to draw conclusions about the effects of dynamic changes and manipulations of transcript levels on protein abundance and is not relevant to the correlation between steady-state levels of mRNA and gene products. Furthermore, the authors are not certain about how the introduction of the antisense constructs is actually working to reduce the presence of the particular transcripts in question: “the antisense constructs... *appeared* to work as siRNAs...” (pg. 2335, emphasis added). In any case, laboratory data from cells genetically manipulated with non-native, unnaturally occurring sequences, which were packaged into expression vectors with foreign sequence elements and produced effects from uncertain subcellular mechanisms, has no relevance to Applicants’ instant invention.

The PTO cites particular observations made in Waghray *et al.* to support its argument that mRNA levels are not necessarily predictive of protein levels, even when there is a change in mRNA levels. Waghray *et al.* looked at transcriptosomal and proteosomal changes in an androgen-sensitive prostate cancer cell line after the cells were treated with dihydrotestosterone (DHT). Out of 16,570 genes, the authors found 351 transcripts that were differentially expressed in the stimulated cells. The authors also identified 44 proteins, out of 1031 spots on protein gels, that were upregulated or downregulated in stimulated cells. Hence, Waghray *et al.* found that over 4% (44/1031) of the proteins isolated from the cells were differentially expressed while only 2% (352/16,570) of the transcripts were differentially expressed.

The PTO posits that if changes in protein generally reflected mRNA changes, one would only expect to see 2% of the proteins differentially expressed, i.e. 22 out of 1031 proteins,

instead of the observed 44 proteins. However, the PTO's conclusion requires that the set of 1,031 proteins found in the protein gels be a proportional, representative, randomized subset of the 16,570 genes found in the analysis of the transcripts. It is clear that the 1031 proteins found represent only a small subset of the 16,570 transcripts examined. The authors stated that "[a] relatively small set of genes could be analyzed at the protein level, largely due to the limited sensitivity of 2-D PAGE" (pg. 1337, emphasis added). Thus, similar to the results of Nagaraja *et al.* discussed *supra*, proteins found through the use of protein gels represent only the most abundant proteins of the cell, whereas the transcripts identified included transcripts of many proteins not abundant enough to be found via protein gels. Because the set of proteins identified do not represent a randomized subset of the transcripts identified, one cannot draw conclusions regarding the general relationship between changes in mRNA levels and changes in protein levels based on a comparison of these data.

The PTO also cites an additional statement by Waghray *et al.* in support of its argument that mRNA levels are not necessarily predictive of protein levels, even when there is a change in mRNA levels. Waghray *et al.* found that corresponding SAGE (sequence analysis) data were available for a number of the proteins identified as differentially expressed and stated that "remarkably, for most of the proteins identified, there was no appreciable concordant change at the RNA level (table 4)." *Final Office Action* at 7. The PTO concludes from this statement that the data presented by Waghray *et al.* support its argument against a correlation between mRNA levels and protein levels.

However, further analysis of the data collected in these experiments shows that such a conclusion cannot be drawn from the data. The experiments of Waghray *et al.* that produced the data shown in Table 4 involve hormonally stimulating cells for 24 hours; determining mRNA levels in the cells; and, 48 hours after determining mRNA levels, determining protein levels, for specific mRNA/protein product pairs. The authors measured mRNA levels twice, before stimulating with DHT and after stimulating with DHT for 24 hours (24 hours post-treatment). They also measured protein concentrations twice, before stimulating with DHT and at 72 hours post-treatment. The second measurement of protein levels therefore occurred 48 hours after DHT was removed from the culture media. Thus, the experiment involved creating a dynamic and changing environment for cells and the measurement of the effects of this changing

environment at only one timepoint. Additionally, the timepoints used for measuring the effects on mRNA levels and protein levels were 48 hours apart.

Examining the two timepoints for particular genes, the authors stated that there was not appreciable concordant change at the RNA level for most of the proteins whose concentrations were affected by DHT treatment. However, the differential expression of mRNA at 24 hours and of protein at 72 hours does not reveal the complete picture of the effects of DHT treatment on the cells. The authors noted that the dynamic conditions of the experiments created fluctuating levels of both mRNA and protein over time (pg. 1337). They decided to examine the kinetics of mRNA and protein levels for two proteins affected by DHT treatment, PSA and clusterin (Fig. 1C on pg. 1334). PSA is known to be an androgen-regulated gene and the authors had been surprised to see only a 1.7 fold induction of PSA transcripts by DHT treatment at the 24 hour timepoint. But through the kinetic experiment, they saw that induction of PSA began between 4 and 6 hours post-treatment and they detected a 5 to 10 fold induction of PSA at 6 to 8 hours post-treatment. PSA mRNA levels subsequently declined, so that by the time samples were taken for SAGE analysis at 24 hours post-treatment, only a 1.7 fold induction was seen. The results of the clusterin kinetic experiment show an even greater effect of DHT treatment on induction and greater fluctuation ranges. Clusterin mRNA induction began sooner than PSA induction (only 0.5 to 1 hour post-treatment), declined between 6-12 hours post-treatment, and at the 24 hour timepoint clusterin mRNA levels had declined to a lower level than the untreated control cells. Thus, while clusterin mRNA was initially induced to much higher than steady-state levels by DHT treatment, by the time the researchers quantified the levels of clusterin mRNA with SAGE at the 24 hour timepoint, clusterin mRNA levels had fallen *below* the levels measured pre-treatment. Due the dynamic nature of these stimulation experiments, it is clear that the observed effect of DHT treatment on the mRNA level of an affected gene will depend on *when* the observation is made. For example, with clusterin, one could observe a large induction of transcription (1-6 hours post-treatment), no change in mRNA levels (some point between 12 and 24 hours post-treatment), or a reduction *below untreated levels* of mRNA (24 hours post-treatment), all depending on the particular timepoint chosen for the collection of an RNA sample from treated cells. Because of these fluctuations of mRNA levels over time, the data from Table 4 have no relevance to the relationship between steady-state levels of mRNA and protein for a

particular gene and cannot inform us as to the general relationship between mRNA levels and protein levels. This is especially true since the authors did not perform kinetic experiments on proteins affected by DHT treatment; it is unknown whether reduced levels of expression seen for some proteins in the table represent a persistent suppression of protein expression over a 72 hour period or merely a reduced level at just the 72 hour timepoint. Thus, the data from Table 4, upon which the authors base their observation about the concordance of mRNA and protein levels, actually provide no insight into the relationship between mRNA levels and protein levels in a dynamic experiment with stimulated cells, let alone for cells in a steady-state environment.

The PTO has cited the observations of Waghray *et al.* regarding their experiments on stimulated cells in support of its argument that mRNA levels are not necessarily predictive of protein levels, even when there are changes in the mRNA level. But because of the differences in transcript and protein detection efficiency and the dynamic nature of the stimulation experiments, no correlations between transcript and protein levels can be accurately drawn from the data presented. The conclusions of the authors have no relevance to and do not support the PTO's argument.

The PTO also cites the work of Sagynaliev *et al.* to support its argument that mRNA levels are not predictive of protein levels, even when considering changes in mRNA levels. The Sagynaliev *et al.* reference is a review of scientific papers regarding gene expression in colorectal cancer (CRC) and describes an attempt by the writers to create a "data warehouse" combining the results of multiple researchers and laboratories into one database. The authors present statistics about how many genes have been found to be differentially expressed at the mRNA level versus at the protein level in CRC studies. The PTO points to these statistics as evidence of the discordance between mRNA and protein levels, noting that while 982 genes were found to be differentially expressed in human CRC by genome-wide transcriptomics technologies, only 177 have been confirmed using proteomics technologies.

The work of Sagynaliev *et al.*, however, does not support the PTO's argument. In their conclusions, the authors are not suggesting that mRNA levels, changing or otherwise, are not predictive of protein levels. Instead, they see the disagreement between different studies, laboratories and experimental techniques as evidence that there is a great need for standardization in this research field: "Thus, the development of standardized processes for collecting samples,

storing, retrieving, and querying gene expression data obtained with different technologies is of central importance in translational research” (pg. 3066).

Far from supporting the PTO’s argument, the research of Sagynaliev *et al.* actually provides a list of problems with the research in the field which serve to reduce the reproducibility of the experiments and thus make conclusions drawn from comparison of experimental results less reliable. Three of the problems listed by the authors serve to undermine the PTO’s use of the data discussed *infra* in support of his argument. First, multiple factors can affect the outcome of a microarray experiment used to analyze a transcriptosome, including technical, instrumental, computational and interpretative factors. The authors found that when comparing different microarray experiments on CRC samples, only four of 185 genes selected behaved consistently on three array platforms and the agreement on the results from two brands of microarray was only about 30% (pg. 3077). Second, in proteomic studies, protein gels have well-known technological limitations, so that even under well-defined experimental conditions, 2-D PAGE analysis is “hampered by a significant variability” (pg. 3077). Third, because of “small sample size (number of patients), large number of variables examined at once, and absence of double or triple experiments (arrays and gels are expensive and samples are rare), statistical analysis is often *not valid*” (pg. 3077, emphasis added). Thus, reproducibility between transcriptosome analysis experiments or between proteosome analysis experiments is hampered by both the lack of technical standardization and the inherent variability of microarray and protein gel technologies. If the reproducibility of experimental results within particular areas of research is questionable, it is unlikely that conclusions drawn by comparing experiments between these research areas (e.g., examining *different* molecular populations) would be valid.

The PTO cites the studies of Nagajara *et al.*, Waghray *et al.* and Sagynaliev *et al.* to support its argument that mRNA levels are not necessarily predictive of protein levels, even when there are changes in the mRNA level. However, any conclusions drawn from the studies of Nagajara *et al.* and Waghray *et al.* are hobbled by the lack of comparability between experiments examining transcriptosomes and proteosomes, as well as the dynamic nature of mRNA levels in stimulated cells. Sagynaliev *et al.* details the problems with reproducibility in the translational research field and hence actually undermines the PTO’s efforts to draw conclusions about mRNA and protein levels by comparing experiments examining different types of molecules. Together

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or separately, these references do not support the PTO's arguments. Applicants therefore respectfully request that the objections to Claims 6-17 based on these references be withdrawn.

Previously Submitted Exhibits 2-13 Are Relevant to the PTO's Argument Against Allowance of the Claims

Applicants continue to assert that it is well-established in the art that a change in the level of mRNA encoding a particular protein generally leads to a corresponding change in the level of the encoded protein; given Applicants' evidence of differential expression of the mRNA for the PRO1268 polypeptide in kidney tumors, it is more likely than not that the PRO1268 polypeptide is also differentially expressed; and proteins differentially expressed in certain tumors, and antibodies that bind such proteins, have utility as diagnostic tools.

Applicants previously submitted exhibits, comprising 81 references, in support of their argument for the correlation between mRNA levels and protein levels. Applicants maintain that the overwhelming evidence they have provided strongly supports Applicants' position.

The PTO also states that "the specification of the instant application does not teach a change in mRNA level of PRO1268. The specification simply discloses a static measurement of PRO1268 PCR in kidney tumor as compared to control. There are no teachings in the specification as to the differential expression of PRO1268 mRNA in the progression of kidney cancer or in response to different treatments of hormones (for example)." *Final Office Action* at 8. Applicants submit that the PTO has misinterpreted the data presented in Example 18. Applicants measured mRNA expression in normal kidney tissue as compared to mRNA expression in cancerous kidney tissue. They found that the mRNA was expressed significantly higher in kidney tumor tissue than in normal kidney tissue. Thus, Applicants discovered a change in mRNA expression: it was significantly more highly expressed in kidney tumor than in normal kidney tissue. This differential expression of the mRNA for PRO1268 provides a specific and substantial utility for the encoded protein.

The PTO also states that Applicants' exhibits, with the exception of Orntoft *et al.* (Mol. Cell. Proteomics, (2002) 1:37-45), "measure mRNA, while the assay utilized in Example 18 of the instant specification is PCR." *Final Office Action* at 4. Again, the PTO appears to misunderstand Applicants data. Example 18 uses PCR to measure mRNA. Thus, the PTO

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statement is nonsensical, and provides no basis to question the relevance of Applicants' previously submitted references.

In support of their arguments, Applicants have previously submitted the Polakis Declaration in support of their position that in general, changes in mRNA levels correlate with changes in protein levels. Applicants submit herewith as Exhibit 1 a second Declaration by Dr. Polakis (Polakis II) that presents evidentiary data in Exhibit B. Exhibit B of the Declaration identifies 28 gene transcripts out of 31 gene transcripts (i.e., greater than 90%) that showed good correlation between tumor mRNA and tumor protein levels. As Dr. Polakis' Declaration (Polakis II) says "[a]s such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is a very strong correlation between increases in mRNA expression and increases in the level of protein encoded by that mRNA." Accordingly, Dr. Polakis has provided the facts to enable the Examiner to draw independent conclusions.

Applicants also submit herewith a copy of a declaration by Randy Scott, Ph.D. (attached as Exhibit 2). Dr. Scott is an independent expert in the field of molecular diagnostics, with over 15 years experience. He is the author of over 40 scientific publications in the fields of protein biology, gene discovery, and cancer, and is inventor on several issued patents. His curriculum vitae is attached to the declaration. In paragraph 10 of his declaration, Dr. Scott states:

One reason for the success and wide-spread use of the DNA microarray technique, which has led to the emergence of a new industry, is that generally there is a good correlation between mRNA levels determined by microarray analysis and expression levels of the translated protein. Although there are some exceptions on an individual gene basis, it has been a consensus in the scientific community that elevated mRNA levels are good predictors of increased abundance of the corresponding translated proteins in a particular tissue. Therefore, diagnostic markers and drug candidates can be readily and efficiently screened and identified using this technique, without the need to directly measure individual protein expression levels. *Scott Declaration* at ¶10 (emphasis added).

Applicants submit the opinion of yet another expert in the field that changes in mRNA level for a particular protein in a given tissue generally lead to a corresponding change in the level of the encoded protein. Importantly, Dr. Scott also states that, contrary to the contentions of the PTO, diagnostic markers can be identified "without the need to directly measure individual protein expression levels." This opinion is supported by Dr. Scott's extensive experience in the field, as

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well as the fact that an entire industry has developed around technology used to assess differential mRNA expression. As stated previously, there would be little reason to study changes in mRNA expression levels if those changes did not result in corresponding changes in the encoded protein levels.

The case law has clearly established that in considering affidavit evidence, the PTO must consider all of the evidence of record anew. *In re Rinehart*, 531 F.2d 1084, 189 USPQ 143 (C.C.P.A. 1976) and *In re Piasecki*, 745 F.2d. 1015, 226 USPQ 881 (Fed. Cir. 1985). “After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of the evidence with due consideration to persuasiveness of argument.” *In re Alton*, 37 U.S.P.Q.2d 1578, 1584 (Fed. Cir. 1996)(quoting *In re Oetiker*, 977 F.2d 1443, 1445, 24 U.S.P.Q.2d 1443, 1444 (Fed. Cir. 1992)). Furthermore, the Federal Court of Appeals held in *In re Alton*, “We are aware of no reason why opinion evidence relating to a fact issue should not be considered by an examiner.” *Id.* at 1583. Applicants also respectfully draw the PTO’s attention to the Utility Examination Guidelines which state, “Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered.” Part IIB, 66 Fed. Reg. 1098 (2001).

In summary, Applicants have submitted herewith two additional expert Declarations in addition to the declarations and over 115 references already of record, which support Applicants’ asserted utility, either directly or indirectly. This evidence overwhelmingly supports the assertion that in general, a change in mRNA expression level for a particular gene leads to a corresponding change in the level of expression of the encoded protein. As Applicants have previously acknowledged, the correlation between changes in mRNA level and protein level is not exact, and there are exceptions. However, Applicants remind the PTO that the asserted utility does not have to be established to a statistical certainty, or beyond a reasonable doubt. *See M.P.E.P.* at § 2107.02, part VII (2004). Therefore, the fact that there are exceptions to the correlation between changes in mRNA and changes in protein does not provide a proper basis for rejecting Applicants’ asserted utility. Applicants submit that considering the evidence as a whole, with the

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overwhelming majority of the evidence supporting Applicants' asserted utility, a person of skill in the art would conclude that Applicants' asserted utility is "more likely than not true." *Id.*

The PTO's Position is Inconsistent with the Utility Guidelines and the Courts

In response to Applicants' evidence and arguments, the PTO takes the position that Applicants must present specific evidence directly demonstrating the utility of the claimed antibodies – specifically, direct evidence of differential expression of PRO1268 polypeptide in tumor and normal tissue. Applicants submit that this requirement is inconsistent with the Utility Guidelines and the courts.

Adopting the PTO's standard for utility would result in a per se rule that a difference in mRNA expression cannot establish a utility for the encoded polypeptide and antibodies thereto. Thus, the PTO chooses to heighten the utility requirement to require specific, direct evidence of utility when there are exceptions to a generally accepted rule that is relied upon for utility. This heightened utility requirement is inconsistent with the Utility Guidelines and the courts. There is no requirement that utility be dispositively proven:

Furthermore, the applicant does not have to provide evidence sufficient to establish that an asserted utility is true "beyond a reasonable doubt." *In re Irons*, 340 F.2d 974, 978, 144 USPQ 351, 354 (CCPA 1965) ... Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true. *M.P.E.P.* 2107.02 VII (emphasis in original).

There is no requirement that only direct evidence of utility is sufficient to establish utility. Instead, it is established that indirect evidence that is reasonably indicative of utility is sufficient to fulfill the requirements of 35 U.S.C. §101. *Nelson v. Bowler*, 626 F.2d 853, 856. Furthermore, there is no requirement that indirect evidence necessarily and always prove actual utility. Instead, there only need be a reasonable correlation between the indirect evidence and the asserted utility. *Id.* at 857, *Cross v. Iizuka*, 753 F.2d 1040, 1050-1051. The indirect evidence need not absolutely prove the asserted utility. All that is required is that the tests be reasonably indicative of the asserted utility. In other words, there need only be a sufficient correlation between the indirect evidence and the utility so as to convince those skilled in the art, to a

reasonable probability, that the novel compound will possess the asserted utility. *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1564.

In the present case, Applicants submit that their evidence (differential mRNA expression) is reasonably linked to the asserted utility (diagnostic use of the encoded polypeptide and specific antibodies). Insofar as it is uncontested that differential mRNA expression is reasonably linked to differential polypeptide expression, Applicants submit that such linkage is sufficient to fulfill the requirements of 35 U.S.C. §101 as provided by the guidance of the Utility Guidelines and the courts.

In conclusion, the PTO's heightened requirement for establishing utility of the presently claimed polypeptides is contrary to the Utility Guidelines and the courts: it is sufficient to present evidence of differential mRNA expression since it is understood in the art that differential mRNA expression is reasonably linked to differential polypeptide expression. As discussed above, even if the PTO has presented evidence that changes in mRNA expression is not always correlated with changes in protein expression, Applicants' overwhelming rebuttal evidence is more than sufficient to establish that changes in mRNA level typically lead to corresponding changes in protein level. As such, Applicants have established that it is more likely than not that one of skill in the art would believe that because the PRO1268 mRNA is differentially expressed in kidney tumors as compared to normal kidney tissue, the PRO1268 polypeptide will likewise be differentially expressed in these tumors. Accordingly, when the evidence is applied to the proper standard for utility, it is clear that this differential expression of the PRO1268 polypeptide establishes the utility of the polypeptide and antibodies that bind the polypeptide as diagnostic tools for cancer, particularly kidney tumor. In view of the above, Applicants respectfully request that the PTO reconsider and withdraw the utility rejection under 35 U.S.C. §101.

Conclusion

The PTO has asserted that the state of the art is such that polypeptide levels cannot be accurately predicted from mRNA levels. Applicants have addressed each of the PTO's supporting references and shown that they are either irrelevant, or taken as a whole, actually support Applicants' assertion that a change in mRNA level leads to a corresponding change in the level of the encoded protein. In addition, Applicants have submitted expert declarations,

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textbook excerpts, and over 115 scientific publications which support Applicants' asserted utility.

Given the totality of the evidence provided, Applicants submit that they have established a substantial, specific, and credible utility for the claimed polypeptides as diagnostic tools. According to the PTO Utility Examination Guidelines (2001), irrefutable proof of a claimed utility is not required. Rather, a specific, substantial, and credible utility requires only a "reasonable" confirmation of a real world context of use. Applicants remind the PTO that:

A small degree of utility is sufficient . . . The claimed invention must only be capable of performing **some** beneficial function . . . An invention does not lack utility merely because the particular embodiment disclosed in the patent lacks perfection or performs crudely... A commercially successful product is not required... Nor is it essential that the invention accomplish all its intended functions... or operate under all conditions... partial success being sufficient to demonstrate patentable utility... In short, **the defense of non-utility cannot be sustained without proof of total incapacity**. If an invention is only partially successful in achieving a useful result, a rejection of the claimed invention as a whole based on a lack of utility is not appropriate. M.P.E.P. at 2107.01 (underline emphasis in original, bold emphasis added, citations omitted).

Applicants submit that they have established that it is more likely than not that one of skill in the art would reasonably accept the utility for the claimed PRO1268 polypeptides set forth in the specification. In view of the above, Applicants respectfully request that the PTO reconsider and withdraw the utility rejection under 35 U.S.C. §101.

Rejections under 35 U.S.C. § 112, first paragraph – Enablement

The PTO maintains its rejection of Claims 6-17 as lacking enablement. The PTO states that because the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility, one skilled in the art would not know how to use the claimed invention.

Applicants submit that in the discussion of the 35 U.S.C. § 101 rejection above, Applicants have established a substantial, specific, and credible utility for the claimed polypeptides. Applicants respectfully request that to the extent the enablement rejection is based on a lack of utility, the PTO reconsider and withdraw the enablement rejection under 35 U.S.C. §112.

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The PTO also states that even if the claimed invention is deemed to have a credible, specific and substantial asserted utility or a well established utility, Claims 6-17 would remain rejected.

As an initial matter, Applicants object to the characterization of its previous response by the PTO: "Applicants did not present further arguments related to Enablement of the claimed invention, stating that Enablement in this case is dependent on the Utility of the invention (11 April 2006, p. 26)." *Final Office Action* at 17. In its previous response, on page 26, Applicants submitted the following language: "Applicants respectfully request that to the extent the enablement rejection is based on a lack of utility, the PTO reconsider and withdraw the enablement rejection under 35 U.S.C. §112." *Response dated April 6, 2005* at 26 (emphasis added). Applicants note that the PTO failed to set forth any arguments in the last four Office Actions issued supporting its position that the claims lack enablement that were not based on the lack of utility arguments. Thus, Applicants did not see the need to address the enablement issue beyond what was submitted in its responses. Nevertheless, Applicants submit the following arguments in support of its position that the claims are fully enabled.

Applicants submit that Claims 6-13 are fully enabled. The scope of these claims is narrow; they do not recite percent amino acid sequence identity as a limitation. These claims are directed to peptides of the disclosed sequence, with or without the disclosed signal peptide, the extracellular domain of the disclosed sequence with or without the disclosed signal peptide, and fusion proteins thereof which would be optimal, for example, in making antibodies. Because SEQ ID NO:90, the signal peptide, the extracellular domain and ATCC deposit 203236 are explicitly disclosed in the specification, no experimentation of any kind is required to make the claimed polypeptides. One of skill in the art would clearly be able to use these polypeptides to make antibodies which are specific to any of these polypeptides, such that the level of expression of these polypeptides could be assessed in kidney tissue. The only question is whether the use of these polypeptides to make antibodies to detect their expression level is a substantial and specific utility. For the reasons discussed at length above, Appellants believe that differential expression of the PRO1268 mRNA in kidney tumors provides the claimed polypeptides with a substantial and specific utility. Therefore, Claims 6-13 are enabled.

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With respect to Claims 14-17, which recite the limitation “wherein said isolated polypeptide or a fragment thereof can be used to generate an antibody which can be used to specifically detect the polypeptide of SEQ ID NO:90 in kidney tissue samples,” Applicants submit that the standard for determining whether the specification meets the enablement requirement is to be evaluated based on whether or not the experimentation needed for one skilled in the art to practice the invention would be undue. *Mineral Separation v. Hyde*, 242 U.S. 261, 270 (1916); *In re Wands*, 858 F.2d 731, 737, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988); *M.P.E.P.* § 2164.01. Applicants submit that in view of the requirements of enablement under 35 U.S.C. §112, first paragraph, the PTO has failed to establish a *prima facie* basis for rejecting Claims 14-17 as lacking enablement. The PTO fails to establish a reasonable basis to question the enablement provided for the claimed invention. *See M.P.E.P.* § 2164.04.

The M.P.E.P. states that “if any use is enabled when multiple uses are disclosed, the application is enabling for the claimed invention,” *M.P.E.P.* § 2164.01(c) (emphasis added). As described below, the specification adequately discloses how to make and use the polypeptides of Claims 14-17. The subject matter of Claims 14-17 relates to isolated polypeptides with at least 95% identity to the disclosed polypeptides wherein the isolated polypeptide or a fragment thereof can be used to generate an antibody which can be used to specifically detect the polypeptide of SEQ ID NO:90 in kidney tissue samples.

The specification teaches in detail how to make the claimed polypeptides, including variants thereof, and antibodies which specifically bind PRO1268. *See, e.g.*, ¶¶ [0283]-[0315]; [0256]-[0271]; [0361]-[0379]; and Examples 6-10 (¶¶ [0453]-[0499]). In addition, the specification discloses that antibodies to claimed polypeptides can be used in diagnostic assays to detect the expression of PRO1268 in specific types of tissue. *See e.g., Specification* at [0407].

Thus, there is significant guidance on how to make and use the claimed polypeptides. In addition, as the disclosure and references cited in the specification make clear, the production of polypeptides, polypeptide variants, and specific antibodies is a predictable and well established aspect of the biological sciences. *See, e.g., In re Wands*, 858 F.2d 731, 8 U.S.P.Q. 2d 1400 (Fed. Cir. 1988) (reversing the Board’s decision of non-enablement and holding that as of 1980, undue experimentation was not required to make high-affinity monoclonal antibodies to a target peptide); *Sutcliffe et al.*, *Science* (1983) 219:660-666 at 661-662 (teaching that “by following

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simple rules, one can in general select peptides that will elicit antibodies reactive with intact proteins”).

In conclusion, the PTO's rejection based on lack of utility has been addressed above, and the PTO has otherwise failed to meet its burden to establish a reasonable basis to question the enablement provided for the claimed invention. Given the skill in the art and the disclosure of how to make and use the claimed polypeptides, Applicants request that the PTO reconsider and withdraw its rejection under 35 U.S.C. § 112, first paragraph.

CONCLUSION

In view of the above, Applicants respectfully maintain that claims are patentable and request that they be passed to issue. Applicants invite the Examiner to call the undersigned if any remaining issues may be resolved by telephone.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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Dated: Oct. 18, 2006

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